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# An Overview of Mitochondrial Toxicity of Nucleoside Reverse Transcriptase Inhibitors Associated with HIV Therapy

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**Abstract:** Mitochondria provide energy required for normal cell function and thus tissue function by producing Adenosine Triphosphate (ATP) via oxidative phosphorylation. They also regulate a number of other cellular processes, including apoptosis. Nucleoside Reverse Transcriptase Inhibitors (nRTIs) remain the cornerstone of Highly Active Antiretroviral Therapy (HAART) combination regimens. However, it has been known for some time that these agents have the potential to cause varied side effects, many of which are thought to be due to their effects on mitochondria. nRTIs can affect the function of this enzyme and this may lead to depletion of mitochondrial DNA or qualitative changes. Various clinical and *in vitro* studies have shown that nRTIs are associated with mitochondrial dysfunction in different tissues, although the weight of evidence is limited in many cases. The heterogeneity in the tissues affected by the different drugs raises interesting questions and possible explanations include differential distribution or activation of these agents. This study gives an overview of the major recognised toxicities associated with nRTI use in Human Immunodeficiency syndrome (HIV) therapy and evidence for mitochondrial dysfunction in these complications.

**Key words:** Mitochondrial toxicity, nucleoside reverse transcriptase inhibitors, HIV therapy

## INTRODUCTION

Mitochondria provide energy required for normal cell function and thus tissue function, by producing Adenosine Triphosphate (ATP) via oxidative phosphorylation[1,2]. They also regulate a number of other cellular processes including apoptosis. Mitochondria are present in all cells except erythrocytes, in numbers of 1 to more than 1000[3,4]. The number of mitochondria correlates with the degree of cellular and tissue metabolic activity. Mitochondria have their own DNA, likely reflecting a bacterial origin and endocytosis by primitive eukaryotic cells during early evolution[5,6].

Mitochondria have also been shown to form a highly integrated network and to undergo what appears to be a frequent process of fusion and fission (Fig. 1)[7,8]. In addition, depletion in mitochondrial DNA has been shown to cause morphological changes in mitochondria from cultured human cells and a high energy demand or oxidative stress will induce proliferation of the mitochondrial network to satisfy the cell's energy needs[9-11].

The main function of mitochondria is to produce energy for the cell in the form of Adenosine Triphosphate (ATP), via the process of oxidative phosphorylation[12,13]. Acetyl-CoA is generated either via glycolysis in the cytosol or ß-oxidation of fatty acids in the mitochondria. The passage of acetyl-CoA through the tricarboxylic acid cycle generates NADH and $FADH_2$, which are powerful reducing agents[7,13]. Oxidative phosphorylation takes electrons from these reducing agents and passes them down the electron transport chain, eventually reducing oxygen to water[14]. The transport of electrons down the different components of the electron transport chain also leads to the pumping of protons out of the mitochondria[5,9]. This creates an electrochemical gradient leading to the return of protons into the mitochondria via specific channels. As protons pass through this channel, an integral component catalysis the synthesis of ATP[15,16]. The ATP is then exchanged with ADP from the cytosol by a specific carrier, the ADP/ATP translocator. In addition, while considering mitochondrial function it is important to acknowledge that mitochondria are known to participate in other cellular processes, particularly apoptosis[17]. Thus, it can be seen that mitochondria are not only essential for energy generation within the cell but also function as key regulators of cellular survival[7,18].

**Mitochondria genome:** Mitochondria have their own DNA, likely reflecting a bacterial origin and endocytosis

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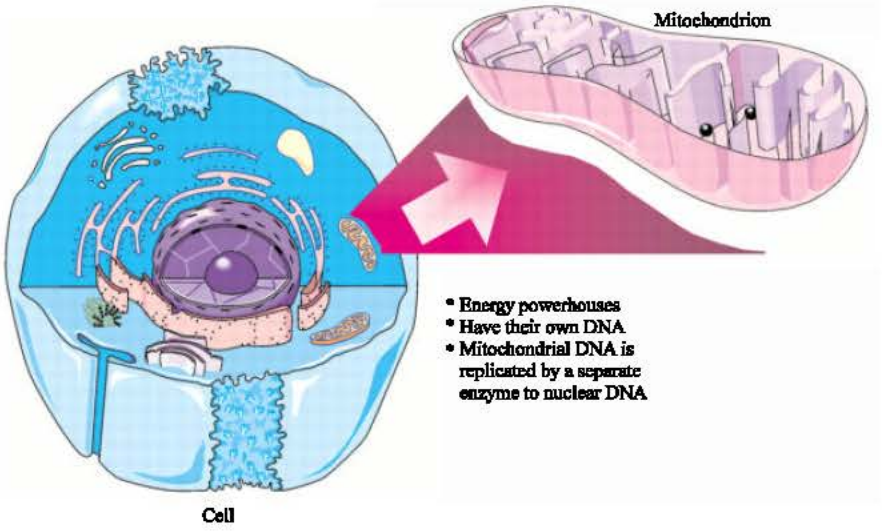


Fig. 1: Ultra structure of Mitochondria and the cell[7]

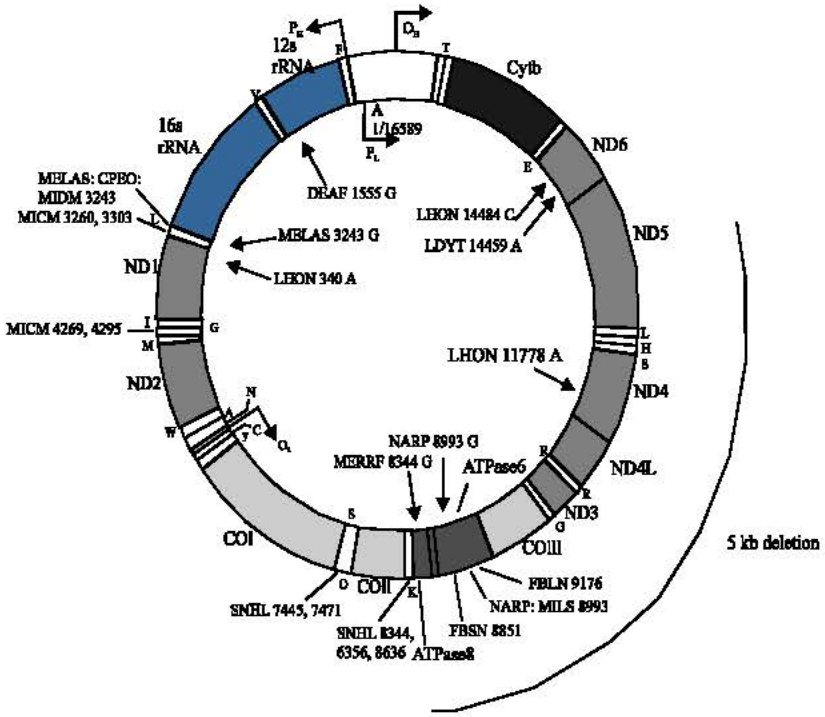


Fig. 2: Human mitochondrial genome. Human mitochondrial genome displaying the 22 tRNA genes (white), two ribosomal RNA genes (yellow) and genes encoding 13 polypetides for complex I: blue, complex III pink, complex IV, red and ATP synthetase (green). The positions of various point mutations and the 5 kb deletion are indicated. Source: MITOMAP: (http://www.gen.emory.edu/mitomap.html)

by primitive eukaryotic cells during early evolution[13]. Mitochondrial DNA is a circular, double stranded DNA molecule of about 16, 000 bases, coding for 13 polypeptides, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs), some of which are key proteins in oxidative phosphorylation (Fig. 2)[10,19]. The gene products of mitochondrial DNA are quite limited and the bulk of the organelle is actually encoded for by nuclear

DNA (nDNA)[7,18]. Considering mitochondrial genetics, there are certain features that are highly significant. Firstly, mitochondrial DNA is maternally inherited. Paternal mtDNA copy number in sperm cells is low in number by comparison with the large number of mtDNA molecules in the oocyte[13]. In addition, it appears that although paternal mtDNA is transferred during fertilisation, it is lost early in embryogenesis. One of the most important observations of mitochondrial genetics is that different mtDNA variants may coexist in a single cell, the state of heteroplasmy[20]. It has also been noted that there is a genetic bottleneck in mitochondrial DNA at some point between oogenesis and development of the embryo[21]. This means that although there may be a large degree of heteroplasmy in the mother, the restriction and amplification that occurs during the bottleneck results in a very small number of mtDNA variants ultimately populating the embryo[10,22].

**Mitochondria DNA replication and DNA polymerase-γ:** Whereas nuclear DNA (nDNA) is replicated by alpha DNA polymerase, mtDNA is replicated by a gamma polymerase, which has a relatively high error rate and some repair capacity[13,15]. Most mitochondria protein is encoded by nDNA. There are at least nine polymerases involved in the replication and maintenance of cellular DNA; however, only one, DNA polymerase gamma, is responsible for mitochondrial DNA replication[7]. Human mitochondrial DNA polymerase is a family of DNA polymerase and has been cloned and characterised[23]. It has also been shown that polymerase gamma is expressed and translated in cells, which have been depleted of mitochondrial DNA[24]. Polymerase gamma has to perform both replication and repair for mtDNA although for some time it was believed that repair activity was absent[25]. However, polymerase gamma has been shown to participate in base excision repair and other repair proteins have also been shown to be present in mitochondria; thus the once common view that mitochondria had little or no capacity for DNA repair should be reconsidered[26].

After HIV has entered the cell, it is required to integrate with the host cell genome. To do this, it needs to convert single stranded viral RNA into double stranded DNA and this task is performed by the enzyme reverse transcriptase[10]. The nRTIs resemble the natural nucleosides but do not have a free 3` hydroxyl group and thus once they are added to the growing DNA chain, termination occurs[27]. Many nRTIs have been investigated for anti-HIV activity and some knowledge of the structure-activity relation of these agents has been established[28]. Since these drugs resemble natural nucleosides, the potential for inhibition of DNA polymerases exists, although there are sufficient differences between the enzymes to enable selective inhibition to occur[29,30].

**nRTI-related mitochondrial toxicity:** Early studies of nRTIs showed minimal effect on DNA polymerase alpha, the main enzyme responsible for nuclear DNA replication, but polymerase beta and gamma were affected to some degree[31,32]. The clinical significance of the inhibition of DNA polymerase beta is unknown, this enzyme synthesises short sections of DNA as part of a group of enzymes involved in repair. Recently it has been shown that Tat, a gene product of HIV, induces the expression of DNA polymerase β[33]. The effects of nRTIs on polymerase gamma have been studied and it appears logical that inhibition of this enzyme and chain termination would lead to mitochondrial DNA depletion, which upon falling below the critical threshold would lead to insufficient energy generation and subsequent cellular dysfunction[34,35]. In addition to the potential effects of gamma polymerase inhibition, nRTIs may also be associated with oxidative damage to mitochondria[27]. Initial *in vitro* studies with nRTIs examined the toxicity of these agents in murine bone marrow progenitor cells, since the first nRTI licensed for the treatment of HIV, zidovudine, was associated with anaemia and neutropenia in the clinic. These studies showed that zidovudine exhibited toxicity in these models, appearing to confirm what was observed in patients[36,37]. Later studies in neuronal cell models showed that the ddC, ddI and d4T caused toxicity, whereas AZT and 3TC did not, again reflecting what was seen in clinical practice[38]. The recognition that nRTIs may interfere with mitochondrial DNA synthesis led to many studies evaluating these effects *in vitro*, recently reviewed by Kakuda[39]. These studies suggested a ranking of ddC ddI d4T > 3TC > ZDV > ABC for effects on mitochondrial polymerase gamma. Martin and colleagues examined both the inhibition of polymerase gamma and the inhibition of mitochondrial DNA synthesis, since some correlation was expected[40]. Although a similar ranking of the nRTIs for effects on mitochondrial DNA synthesis was noted, there was no clear correlation with the potency of mitochondrial DNA inhibition[37,40].

**Metabolic activation of the nRTIs:** The anabolism of the nRTIs is an important factor of consideration; because these agents need to be phosphorylated three times before they can be added to the growing DNA chain by HIV reverse transcriptase or other polymerases[41,42]. This process is known to vary with the activation state of the cell, with stavudine (d4T) and Zidovudine (ZDV) being

more active in activated cells and other nRTIs being more active in resting cells. In addition, intermediary anabolites may be implicated in toxicity of nRTIs, as has been shown for ZDV monophosphate in a study of CEM cells[43,44]. Considering the mitochondrial effects of nRTIs, it is important to note that many cellular kinases exist in both mitochondrial and cytosolic forms. In addition to their subcellular localisation, these kinases frequently differ in their substrate specificity and regulation through the cell cycle. Early studies of zalcitabine (ddC) suggested that the drug was phosphorylated in the cytosol and then transported into the mitochondria. In the neuronal cell model referred to above, it was noted that ddC was only phosphorylated to the monophosphate in mitochondria, compared with the monophosphate, diphosphate and triphosphate in the cytosol[38].

**nRTI transport system:** The entry of nRTIs into cells has been observed to occur at different rates and there are many transport systems available for nucleosides. Since the phosphorylation of nRTIs may differ between subcellular compartments, it follows that movement of the drugs and their anabolites between the cytosolic and mitochondrial compartments is of considerable interest[41]. Early studies with lamivudine (3TC) showed synergistic or additive activity against HIV *in vitro* and also protection against the delayed mitochondrial toxicity associated with d4T, ZDV, ddC and ddI[45]. The protection conferred by 3TC in this study was thought to be due to interference with the uptake of the other agents into mitochondria. Subsequent experiments have shown that other unnatural nRTIs in the same class, such as L(-)Fd4C, also show similar properties[46]. Considering nRTI transport into mitochondria, the studies discussed above with 3TC and L(-)Fd4C suggest that this process may be inhibited, although whether nRTI anabolites can be exported from mitochondria remains unclear. Further understanding of the activation and transport of the nRTIs within different subcellular compartments may lead to molecules or strategies in which efficacy can be enhanced and toxicity reduced[7,10] .

**Clinical manifestations of nRTI-related mitochondria toxicity:** Studies have shown identification of a potential association between nRTI treatment and mitochondrial toxicity and it was recognized that effects observed in nRTI-treated patients resembled clinical manifestations of inherited mitochondrial diseases[47].

nRTIs are associated with a wide spectrum of toxicities, many also caused or exacerbated by HIV itself. Since the tissue involvement and clinical presentation often resembles aspects of inherited mitochondrial disease and it is known that nRTIs may affect mitochondrial function, many authors have proposed that mitochondrial toxicity of the nRTIs is the underlying pathophysiology behind most of these toxicities[48]. The polymerase gamma hypothesis suggests that the manifestations of nRTI toxicity relate to the combined effects of four principal factors[49]. Firstly, the tissue must have some dependence on oxidative phosphorylation; secondly, the nRTI must pass into the tissue itself; thirdly, the nRTI must be phosphorylated by cellular kinases and finally, it must inhibit polymerase gamma activity by competing with the natural substrate or by chain termination[49].

There is a more fundamental lack of correlation between polymerase gamma inhibition and mitochondrial DNA depletion as identified by Martin and colleagues[40]. In addition, the neuronal cell model showed that the neurotoxic effect of d4T did not correlate with mitochondrial DNA depletion, whereas there was a correlation between the effect of ddC and mitochondrial DNA levels[38]. A full reappraisal of the polymerase gamma hypothesis is beyond the scope of this review, although our extended understanding of the many processes involved in nRTI toxicity warrants some expansion of the initial ideas.

**Haematological toxicity:** There are at least some superficial resemblances of the haematological abnormalities observed in patients with Pearson's marrow-pancrease syndrome to the anaemia and neutropenia associated with zidovudine[49,50]. HIV infection and AIDS are known to be associated with significant haematological toxicity, including anaemia, neutropenia and thrombocytopenia[51]. In addition, studies with zidovudine have shown that this drug may compound the haematological toxicity of HIV and lead to the independent development of anaemia and neutropenia[52]. Consistent with these observations, the incidence of anaemia or neutropenia in mildly or asymptomatic adults treated with zidovudine was between 1.1 and 9.7%, whereas in adults with AIDS or AIDS related complex it ranged from 15% to as high as 61%[53]. This toxicity is generally reversible and may be managed by dose reduction or drug withdrawal. Other interventions also appear to confer varying degrees of benefit, including growth factors for neutropenia[54] and recombinant haemoglobin[55] or erythropoietin[56] for anaemia. *In vitro* studies also confirm the haematological toxicity of HIV and zidovudine[36] although the mechanism of this toxicity is unclear.

**Myopathy:** Both HIV infection and zidovudine treatment have been associated with myopathy at all stages of the disease, with the respective disorders being difficult to

distinguish on a clinical basis[47,57] Distinguishing the disease and drug related myopathies has proved difficult[58]. In AIDS Clinical Trials Group (ACTG) study 016, myopathy developed in 1.8% of patients receiving zidovudine monotherapy at 1200 mg per day.

Studies by Simpson[59] illustrates that drug related myopathy is quite rare, particularly with currently used doses and a number of studies have attempted to explain the pathophysiology of this disorder. Benbrik and colleagues studied the effects of ddI, ddC and ZDV on cultured human muscle cells and showed that although ZDV was the most potent inhibitor of cell proliferation, ddC and ddI were the most potent inhibitors of mitochondrial function[17]. Depletion of mitochondrial DNA has been reported in patients with zidovudine related myopathy and this has been shown to be reversible on drug withdrawal[60].

**Cardiomyopathy:** HIV infection itself has been shown to cause cardiomyopathy. Cardiac muscle has a large energy demand and thus features a large mitochondria population[61]. Abnormal cardiac pathology was identified in postmortem studies of AIDS patients in the mid 1980s and subsequent studies have shown that cardiac involvement in HIV infection is by no means uncommon[62]. It is known that mitochondrial dysfunction may frequently be associated with heart disease[63]. *In vitro* studies have identified that cardiac mitochondrial DNA polymerase may be inhibited by zidovudine,[10,41] and studies in rats have shown that the drug may also induce ultrastructural changes in cardiac myocytes[1,41,64]. Others have reported an association between cardiomyopathy and therapy with ZDV, ddC or ddI[65] but the major part appears to be played by HIV disease or other as yet unidentified factors[3,66]. It has also been suggested that mitochondria dysfunction might contribute to atherosclerosis in HIV infected patients[1,61].

**Hepatic steatosis and lactic acidosis:** Cases of liver failure with macrovesicular and microvesicular steatosis have been reported in association with nRTI therapy[67-69]. It is believed that mitochondria dysfunction in the liver results in inhibition of fatty acid oxidation, resulting in accumulation of triglycerides and fatty acids in vesicles[71,72]. Lactic acidosis is a feature of hepatic steatosis syndrome. Inhibition of oxidative metabolism results in anaerobic metabolism and increased lactate production in cell culture systems[34,72]. The incidence of hepatic steatosis is relatively low (1.3-3.9 cases per 1000 patients-years of nRTI use but the case of fatality is high[70,73,74]. A possible mechanism for nRTI-induced

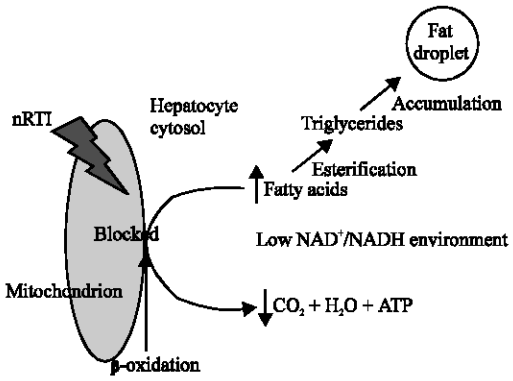


Fig. 3: A possible mechanism for nRTI-induced hepatic steatosis. NAD+ is needed for the catabolism i.e., β-oxidation of fatty acids in hepatocytes. Therefore, its scarcity as a result of nRTI-induced inhibition of oxidative phosphorylation may also underlie nRTI-related hepatic steatosis[11]

hepatic steatosis. $NAD^+$ is needed for the catabolism i.e., β-oxidation of fatty acids in hepatocytes. Therefore, its scarcity as a result of nRTI-induced inhibition of oxidative phosphorylation may also underlie nRTI-related hepatic steatosis[11] (Fig. 3). When a cell is unable to generate enough energy through oxidative phosphorylation, anaerobic respiration occurs via the conversion of pyruvate to lactate in the cytoplasm[75]. This also results in excess production of hydrogen ions, which if uncontrolled, may lead to a cellular and subsequently metabolic acidosis[76,77]. The liver and kidneys normally perform clearance but if the production is excessive or these organs are damaged, accumulation of lactate and hydrogen ions may occur and severe lactic acidosis may result[11,67].

**Lipodystrophy and Lipoatrophy:** The lipodystrophy syndrome and associated metabolic alterations are the most prevalent adverse effects in HIV-infected patients taking Highly Active Anti-Retroviral Therapy (HAART)[78-80]. This syndrome involves profound disturbances in the adipose tissue.

The phenotype of lipoatrophy in HIV-infected patients receiving antiretroviral therapy at least superficially resembles that in Madelung,s disease (multiple symmetric lipomatosis), which is associated with mtDNA mutations[81-83]. It has been hypothesized that mitochondrial toxicity of adipocytes associated with antiretroviral therapy may lead to adipocyte apoptosis and thus lipoatrophy[28,84]. It can be seen that overall

duration of nRTI therapy and d4T therapy in particular are associated with the development of lipodystrophy. Since time on therapy has been identified as a risk factor the association with d4T could simply be because it was the most recently used nucleoside[85,86].

**Osteopenia:** All available antiretroviral agents are associated with significant adverse drug effects. Of particular interest are the newly emerging suspected adverse drug effects, which were not generally noted in pre-marketing trials nor captured under current standard clinical care practice[10,87,88]. Metabolic bone abnormalities such as decreased bone mineral density, osteopenia, osteoporosis and osteonecrosis have been linked to suspected antiretroviral toxicities[69,89]. Osteopenia is commonly used for bones that have become somewhat less dense than normal, but not as severe as in osteoporosis[90-92]. This has been known to be associated with mitochondrial DNA toxicity in patients with HAART therapy, for HIV infection[7,89,91]. A person with osteopenia is at risk for getting osteoporosis, which is most common with women after menopause[68,92-95]. The Caucasian and Asian women are about twice as likely to get osteoporosis as African-American women. However in any given ethnic group, older women have about twice the risk for osteoporosis as older men. It has been hypothesized that osteopenia may result from mitochondrial dysfunction in bones[30,71,96]. Osteopenia has been reported in HCV-infected patients without HIV infection receiving ribavirin/interferon alfa therapy[95,97-99].

**Renal toxicity:** Adefovir, a nucleotide analogue, was shown to induce nephrotoxicity in a significant proportion of patients after longer than 6 months exposure[97,100-103]. Recent experiments have demonstrated that adefovir is a substrate for the human renal organic anion transporter 1 and is thus concentrated in the cells of the proximal tubule[88,99,104]. It has been shown to result in increased cytotoxicity *in vitro*, although the mechanism of this cytotoxicity is unclear[99,100,105]. The clinical presentation of renal involvement in inherited mitochondrial disease is similar to that noted for adefovir toxicity[2,101,106] and adefovir is known to inhibit mitochondrial DNA polymerase[21,102,103], but it is currently unknown whether the nephrotoxicity of adefovir is mitochondrial in nature[103,109,110]. Adefovir is no longer being pursued for the treatment of HIV, although lower doses are being studied for the treatment of hepatitis B infection[7,104-106]. No particular association with other nucleoside analogues has been reported, although a case of nephrotoxicity with ddI was reported some years ago[102,107,108] and one with d4T/3TC more recently[104,110,111] although the latter may have been associated with decompensated hyperlactataemia[111-113].

## CONCLUSIONS

There are many issues remaining to be clarified about the effects of nRTIs on mitochondria and the potential for clinical manifestations of these effects. Some of these issues involve the different adverse effects among nRTIs that may be associated with mitochondrial toxicity for example why is zidovudine associated with myopathy, whereas stavudine is associated with neuropathy. The manifestations of mitochondrial defects are likely to occur in tissue dependent on oxidative phosphorylation and it may be that nRTIs differ in capacity to penetrate different tissues or that cellular kinases that phosphorylate nRTIs, function differently for different drugs and in different tissues. Similarly, there may be some differences among nRTIs regarding the ability of gamma polymerase to proofread and excise the nRTI once it is incorporated into the DNA chain. It still remains unclear whether nRTIs have an additive or synergistic effects on mitochondria when used in combination. It is also not evident why only some patients appear to have mitochondrial toxicity or clinical manifestation of such toxicity. Apart from the potential drug effects, the HIV infection, HCV co-infection, alcohol abuse and genetic factors may play a role in enhancing mitochondria toxicities. The studies on mitochondria DNA polymerase inhibition by nRTIs has helped to explain the toxicities associated with drugs of this class and the data from cell culture, animal and to some extend biopsy studies in human have provided some supporting evidence for this hypothesis.

Continued study into the pathogenesis of nRTI associated toxicity, coupled with the significant advances being made in mitochondrial research, should lead to improved understanding which will facilitate interventions to manage these toxicities and aid the development of newer agents for which these toxicities are absent or minimised.

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